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NOVEL STRUCTURAL DETERMINANTS OF THE
HUMAN NEUTROPHIL CHEMOTACTIC
ACTIVITY OF LEUKOTRIENE B*

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Leukotrienes are a unique class of potent humoral and cellular mediators that are generated by the specific lipoxygenation and subsequent enzymatic conversions of arachidonic acid in basophils, mastocytoma cells, polymorphonuclear leukocytes (PMN) and mononuclear leukocytes, and human lung tissue (1-6). The structural characteristics shared by the leukotriene mediators include three conjugated double bonds, a 5-hydroxy group, and either a 6-sulfido-cysteine(yl) or 12-hydroxy group. 5-hydroxy-6-sulfido-glutathionyl-eicosa-7,9,11,14-tetraenoic acid (leukotriene C₄ [or LTC₄], where the subscript 4 indicates the total number of double bonds) and 5-hydroxy-6-sulfido-cysteinyl-glycine-7,9,11,14-tetraenoic acid (LTD₄), which are the principal active constituents of the slow-reacting substance of anaphylaxis (termed SRS-A), constrict pulmonary airways with a potency up to 20,000 times greater than that of histamine and alter the tone and permeability of the microvasculature (7). 5(S),12(R)-di-hydroxy-eicosa-6,8,10(trans/trans/cis),14(cis)-tetraenoic acid, which is designated LTB₄ (6), is chemotactic and chemokinetic for human and rat PMN and, to a lesser extent, mononuclear leukocytes (8-10). The maximal human neutrophil chemotactic response elicited by LTB₄ is similar in magnitude to those evoked by synthetic formyl-methionyl peptides or fragments of the fifth component of human complement, and the neutrophil chemotactic potency of LTB₄ is far greater than that of the mono-hydroxy-eicosatetraenoic acids (HETE) derived from arachidonic acid, whereas neither LTC₄ nor platelet-derived tri-HETE exhibit leukocyte chemotactic activity (8). The results of our study indicate that the conformation of the hydroxyl-groups and the geometry of the double bonds in the conjugated triene are the critical structural determinants of the potency of LTB₄ as a neutrophil chemotactic factor.

Materials and Methods

Preparation and Purification of Lipoxygenase Products of Arachidonic Acid. Neutrophils that had been isolated from venous blood of normal subjects were incubated for 30 min at 37°C at a concentration of 5×10^7 neutrophils/ml in Hanks' balanced salt solution containing 0.02 M Tris-HCl (pH 8), 10 μ M indomethacin, 5 μ M calcium ionophore A23187, and 0.5 mg/ml of arachidonic acid. The HETE were extracted and resolved from both the residual arachidonic acid and more polar products by silicic acid column chromatography (6, 8). LTB₄ and 5-HETE

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were purified by reverse-phase high performance liquid chromatography (HPLC) on a C18 ODS column (Ultrasphere; Altex Scientific, Inc., Berkeley, Calif.) that was developed isocratically at a flow rate of 1 ml/min with methanol:water:glacial acetic acid (78:22:0.01, vol:vol:vol). The 5,12-di-HETE and 5,6-di-HETE isomers contained in the eluates of the silicic acid column were resolved from LTB₄ and mono-HETE and were partially purified by reverse-phase HPLC on Nucleosil C-18, 5 μ m (Machery-Nagel, Düren, Federal Republic of Germany) for 60 min. The 5,12-di-HETE and the 5,6-di-HETE were purified by rechromatography separately on the Nucleosil column utilizing methanol:water:glacial acetic acid (65:35:0.01, vol:vol:vol) and methanol:water:glacial acetic acid (75:25:0.01, vol:vol:vol), respectively. The highly purified LTB₄ and di-HETE isomers contained <1% total of the other isomers.

The chromatographic characteristics of the two 5,6-di-HETE stereoisomers, for which the conformation of the hydroxyl-groups and the geometry of the double bonds have not been determined, and the two 5,12-di-HETE with 6,8,10(all-trans), 14(cis)-double bonds, termed 5,12-all-trans-di-HETE, have been described previously (6). 5,6-di-HETE-a eluted before 5,6-di-HETE-b from the second Nucleosil C-18 column. The 5,12-di-HETE were converted into dimethoxycarbonyl derivatives, subjected to oxidative ozonolysis, and treated with diazomethane, and the products were analyzed by gas chromatography-mass spectrometry to determine the configuration of the 5- and 12-hydroxy-groups (6). The triene portion of LTB₄ consists of one cis- and two trans- double bonds, but the position of the cis- bond has not been established unequivocally. LTB₄ was methylated by reaction with a 500-fold molar excess of diazomethane in ethyl ether for 10 min at room temperature and was acetylated in pyridine by reaction with a 100-fold molar excess of acetic anhydride for 20 min at room temperature; both procedures were carried out in an argon environment (11, 12). The identity of each of the purified products was confirmed by ultraviolet light absorption spectroscopy, infrared spectroscopy, and gas chromatography-mass spectrometry; the products were quantified by optical density at characteristic wavelengths of maximal absorption (6).

Assessment of human neutrophil migration. Chemotaxis of human neutrophils of >96% purity was assayed by a modification of the Boyden technique using chambers with a 0.2 ml blind-end stimulus compartment (Neuro Probe, Inc., Bethesda, Md.) and filters with 3- μ m pores (Sartorius, Science Essentials Division of Beckman Instruments, Inc., Wakefield, Mass.) (8, 11, 12). Chemotactic fragments of the fifth component of human complement (C5) were prepared as described (11). The chemotactic responses were expressed as net neutrophils/high-power field (hpf) after subtraction of the background level of migration in the absence of a stimulus. In studies designed to examine the inhibition of the neutrophil chemotactic response to LTB₄ by derivatives of LTB₄, the native principle and the derivative were present together in the chemotactic factor compartment and the response to the derivative alone was subtracted from the activity of the mixture. A standard two-sample Student's *t*-test was used to calculate levels of significance.

Results

LTB₄ elicited a neutrophil chemotactic response that was detectable at a concentration of 0.3 ng/ml or $\sim 10^{-9}$ M and was maximal at 30 ng/ml or $\sim 10^{-7}$ M (Fig. 1). The maximal neutrophil chemotactic response to LTB₄ was similar in magnitude to those evoked by the chemotactic fragments of C5 (56.6 ± 7.4 , mean \pm SD, $n = 4$) or by 10^{-8} M formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe) (60.3 ± 9.2). The other di-HETE examined were significantly less potent than LTB₄ as chemotactic stimuli, but at a concentration of 1,000 ng/ml evoked maximal chemotactic responses that were not significantly different from that seen with LTB₄ at 10 ng/ml. Thus LTB₄ was ~ 100 -fold more potent than the other di-HETE, when comparing the concentrations required to stimulate maximal chemotactic responses. An analysis of the concentrations needed to achieve a response equal to 50% of the maximal level indicated that the ratio of potency of LTB₄ to the analogues ranged from 15 for stereoisomer a of the 5,6-di-HETE to >100 for 5(S),12(S)-all-trans-di-HETE. The

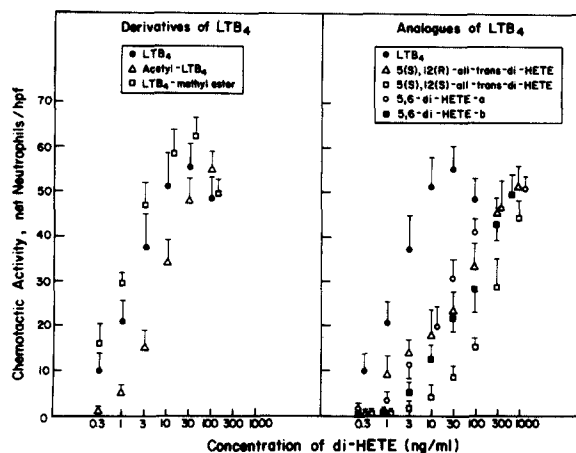


FIG. 1. Human neutrophil chemotactic activity of derivatives and analogues of leukotriene B₄. The points and brackets represent the mean \pm SD of the results of eight determinations of activity for LTB₄, five for acetyl-LTB₄ and four for the other factors. The designation "all-trans" refers to the geometry of only the conjugated double bonds in the triene portion of the molecule. LTB₄ was significantly more chemotactic for neutrophils ($P < 0.01$) than was acetyl-LTB₄ at concentrations of 0.3–10 ng/ml, 5,6-di-HETE-a at 0.3–30 ng/ml, and the other analogues at 0.3–100 ng/ml.

5(S),12(R)-all-trans-di-HETE, in which the 5- and 12-hydroxyl groups possess a different conformation, was substantially more potent than the isomer with optically equivalent 5(S)- and 12(S)-hydroxyl substituents.

Acetylation of LTB₄ decreased the chemotactic activity significantly at concentrations of 0.3–10 ng/ml, but not at maximally active concentrations, while incubation in pyridine alone or methyl esterification had no effect (Fig. 1). In contrast, the results of parallel experiments showed that the methyl ester of 5-HETE had significantly less neutrophil chemotactic activity than 5-HETE at concentrations of 200–5,000 ng/ml, whereas acetyl-5-HETE expressed neutrophil chemotactic activity that ranged from 75–87%, 78–91%, and 82–94% that of 5-HETE at concentrations of 200, 1,000 and 5,000 ng/ml, respectively. The presence of minimally chemotactic concentrations of acetyl-LTB₄ suppressed the chemotactic responses to LTB₄ in proportion to the molar ratio of the factors (Fig. 2), but had no effect on the responses to chemotactic fragments of C5 or to f-Met-Leu-Phe. At an equimolar concentration, acetyl-LTB₄ inhibited by ~50% the chemotactic responses to 1 ng/ml and 4 ng/ml of LTB₄. Incubation of portions of neutrophils with 4 ng/ml and 10 ng/ml of acetyl-LTB₄, followed by washing, failed to suppress the subsequent chemotactic response to 1 ng/ml of LTB₄ relative to neutrophils preincubated in buffer alone. LTB₄-methyl ester had only a minimal inhibitory effect that reached 20% at a 10-fold ratio of LTB₄-methyl ester:LTB₄.

Discussion

LTB₄ elicits a maximal neutrophil chemotactic response and a response that is one-half of the maximal level at concentrations of $\sim 10^{-7}$ M and $\sim 6 \times 10^{-9}$ M, respectively (Fig. 1), which are similar in magnitude to those evoked by 4×10^{-6} M and 4×10^{-7} M 5-HETE (13), and by 1.7×10^{-8} M and 1.3×10^{-9} M C5a (14). The neutrophil chemotactic activity of acetyl-LTB₄ is significantly less than that of LTB₄ at concen-

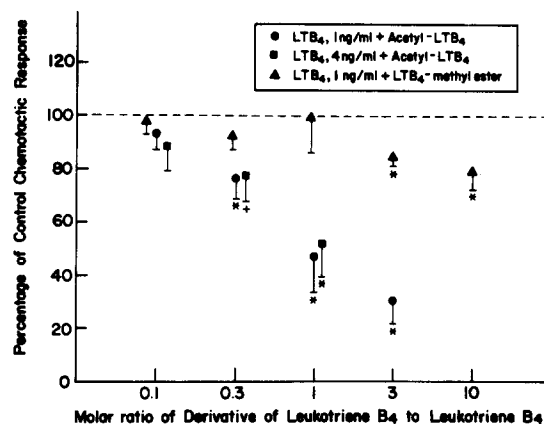


FIG. 2. Inhibition of the human neutrophil chemotactic response to leukotriene B₄ by acetyl-leukotriene B₄. The points and brackets represent the mean \pm SD of the results of four assays with neutrophils from different subjects. The control chemotactic responses (100%) to 1 ng/ml and 4 ng/ml of LTB₄ ranged from 18.4–26.7 and from 33.6–41.9 net neutrophils/hpf, respectively. *, $P < 0.01$; and +, $P < 0.05$.

trations on the ascending portion of the dose-response curve, whereas the chemotactic potency of acetyl-5-HETE and of 5-HETE are similar. Free hydroxyl-group(s) thus are critical determinants of the chemotactic potency of LTB₄, but are not required for the expression of maximal chemotactic activity at higher concentrations. In contrast, methylation of the carboxyl group substantially suppressed the chemotactic potency and maximal activity of 5-HETE, but had no significant effect on the function of LTB₄.

The availability of a series of other highly purified di-HETE, that were generated by neutrophils, permitted an analysis of the functional role of the double bonds in the conjugated triene of LTB₄. The triene of LTB₄, as for that of LTC₄ and LTD₄, consists of one cis- and two trans- double bonds, but the positions of the bonds have not been established unequivocally for LTB₄ (3, 5, 6). The 5(S),12(R)-all-trans-di-HETE isomer, which differs from LTB₄ only in having a trans-double bond in place of a cis-double bond in the triene portion of the molecule (6), is significantly less potent than LTB₄, but evokes a maximal chemotactic response of similar magnitude (Fig. 1). The cis-double bond and the free hydroxyl-group(s) thus appear to be critical determinants of the potency of LTB₄, but are not required to attain maximal activity unlike the free carboxyl-group of 5-HETE. Although the other 5,12-di-HETE and both 5,6-di-HETE isomers also were less potent than LTB₄, differences in the conformation or position of the second hydroxyl-group as well as in the structure of the triene may have contributed to the altered function.

The capacity of minimally chemotactic concentrations of acetyl-LTB₄ to inhibit selectively the human neutrophil chemotactic response to LTB₄ is reminiscent of the competitive inhibition of the chemotactic responses to 5-HETE by 5-HETE methyl ester (9) and to f-Met-Leu-Phe by several chemotactically inactive peptide analogues (15, 16). That the washing of neutrophils that had been preincubated with acetyl-LTB₄ fully reversed the suppression of the chemotactic response to LTB₄ suggests a competitive mechanism of inhibition. The results of competitive binding studies indicate that some inactive peptide analogues inhibit the chemotactic and other

activities of f-Met-Leu-Phe by displacing the stimulus from the receptor, but comparable binding data are not yet available for LTB₄ or the mono-HETE.

Summary

A specific 5(S),12(R)-dihydroxy-eicosa-6,8,10(trans/trans/cis), 14(cis)-tetraenoic acid, designated leukotriene B, is generated by the lipoxygenation and subsequent enzymatic hydration of arachidonic acid in a variety of leukocytes. Leukotriene B elicits a maximal human neutrophil chemotactic response in vitro which is similar in magnitude to those evoked by the chemotactic fragment of the fifth component of complement, C5a, synthetic formyl-methionyl peptides, and 5-hydroxy-eicosatetraenoic acid (5-HETE). The neutrophil chemotactic potency of purified leukotriene B, assessed by the 50% effective concentration of 6×10^{-9} M, is equivalent to that of C5a, but is up to 100-fold greater than that of 5-HETE and of other natural di-HETE isomers. 5(S),12(R)-di-hydroxy-eicosa-6,8,10(all-trans),14(cis)-tetraenoic acid, which differs from leukotriene B only in having a trans-double bond in place of a cis-double bond in the triene portion of the molecule, and acetyl-leukotriene B are significantly less potent neutrophil chemotactic factors than leukotriene B, which indicates that both the conjugated double bonds and the free hydroxyl-group(s) are functionally critical determinants. The capacity of acetyl-leukotriene B to inhibit competitively and selectively the human neutrophil chemotactic response to equimolar concentrations of leukotriene B suggests the existence of a specific subset of receptors for this potent lipid mediator.

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References

1. Goetzl, E. J. 1980. Mediators of immediate hypersensitivity derived from arachidonic acid. *N. Engl. J. Med.* **303**:822.
2. Murphy, R. C., S. Hammarstrom, and B. Samuelsson. 1979. Leukotriene C: a slow reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. U. S. A.* **76**:4275.
3. Parker, C. W., M. M. Huber, M. K. Hoffman, and S. F. Falkenheim. 1979. Characterization of the two major species of slow reacting substance from rat basophilic leukemia cells as glutathionyl thioethers of eicosatetraenoic acids oxygenated at the 5 position: evidence that peroxy groups are present and important for spasmogenic activity. *Prostaglandins*. **18**:673.
4. Orning, L., S. Hammarstrom, and B. Samuelsson. 1980. Leukotriene D: a slow-reacting substance from rat basophilic leukemia cells. *Proc. Natl. Acad. Sci. U. S. A.* **77**:2014.
5. Lewis, R. A., K. F. Austen, J. M. Drazen, D. A. Clark, A. Marfat, and E. J. Corey. 1980. Slow reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. *Proc. Natl. Acad. Sci. U. S. A.* **77**:3710.
6. Borgeat, P., and B. Samuelsson. 1979. Metabolism of arachidonic acid in polymorphonuclear leukocytes. Structure analysis of novel hydroxylated compounds. *J. Biol. Chem.* **254**: 7865.
7. Drazen, J. M., K. F. Austen, R. A. Lewis, D. A. Clark, G. Goto, A. M. Marfat, and E. J. Corey. 1980. Comparative airway and vascular activities of leukotrienes C-1 and D *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* **77**:4354.
8. Goetzl, E. J., and W. C. Pickett. 1980. The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETEs). *J. Immunol.* **125**:1789.
9. Palmer, R. M. J., R. J. Stepney, G. A. Higgs, and K. E. Eakins. 1980. Chemokinetic

- activity of arachidonic acid lipoxygenase products on leucocytes of different species. *Prostaglandins*. **20**:411.
10. Ford-Hutchinson, A. W., M. A. Bray, M. V. Doig, M. E. Shipley, and M. J. H. Smith. 1980. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature (Lond.)*. **286**:264.
 11. Goetzl, E. J., F. H. Valone, V. N. Reinhold, and R. R. Gorman. 1979. Specific inhibition of the polymorphonuclear leukocyte chemotactic response to hydroxy-fatty acid metabolites of arachidonic acid by methyl ester derivatives. *J. Clin. Invest.* **63**:1181.
 12. Valone, F. H., and E. J. Goetzl. 1980. Structural requirements for the activity of an immunologically generated lipid chemotactic factor. *Immunology*. **41**:517.
 13. Goetzl, E. J., A. R. Brash, A. I. Tauber, J. A. Oates, and W. C. Hubbard. 1980. Modulation of human neutrophil function by mono-hydroxy-eicosatetraenoic acids. *Immunology*. **39**:141.
 14. Fernandez, H. N., P. M. Henson, A. Otani, and T. E. Hugli. 1978. Chemotactic response to C3a and C5a. I. Evaluation of C3a and C5a leukotaxis *in vitro* and under simulated *in vivo* conditions. *J. Immunol.* **120**:109.
 15. Williams, L. T., R. Snyderman, M. C. Pike, and R. J. Leftkowitz. 1977. Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1204.
 16. Naccache, P. H., M. Volpi, H. J. Showell, E. L. Becker, and R. I. Sha'afi. 1979. Chemotactic factor-induced release of membrane calcium in rabbit neutrophils. *Science (Wash. D. C.)*. **203**:461.